DIGESTIVE ENZYMES PROFILE IN *OCTOPUS VULGARIS* PARALARVAE FED WITH *ARTEMIA* ENRICHED WITH MARINE PHOSPHOLIPIDS

M.V. Martín*, D. Garrido, L. Luis-Hernández, A. deCos-Gandoy, B.C. Felipe, E. Almansa.

Centro Oceanográfico de Canarias, Instituto Español de Oceanografía, Vía Espaldón, Dársena Pesquera PCL 8, 38180 Santa Cruz de Tenerife, Spain.
E-mail: virginia.martin@ca.ieo.es

Introduction

The common octopus (Octopus vulgaris) is an excellent candidate for aquaculture production however the development of its culture needs to overcome the high paralarvae mortality which points out to zootechnical and nutritional problems. Enhancing the knowledge on paralarvae digestive physiology could increase the possibilities to optimize the diet in order to improve the paralarval growth and survival.

In the present study, the effect of feeding with Artemia enriched with marine phospholipids on digestive enzyme activity of octopus paralarvae from hatchling and 12 days old paralarvae have been studied.

Materials & methods

The paralarvae were obtained from wild adult octopus captured in Tenerife coastal waters and maintained in the facilities of the Oceanographic Center of Canary Island (Spanish Institute of Oceanography). A total of 18000 paralarvae, 3000 per tank (6 paralarvae/L) were reared during 12 days in 500 L cylinder-conical tanks, with a flow-through seawater system. Green-water system was used ($5 \cdot 10^5$ cell/mL *of Nannochloropsis sp*, Phytobloom Green Formula®, Portugal). Paralarvae were fed with *Artemia* (Sep-Art AF, INVE Aquaculture, Belgium) enriched: control group with phytoplankton (*Isochrysis aff. galbana* T-Iso, supplied by easy algae®, Cádiz, Spain) *and Nannochloropsis sp)* and experimental group with Marine Lecithin LC 60® (PhosphoTech Laboratoires, France) (LC60). Each treatment was carried out in triplicates. *Artemia* was supplied 3 times a day at 0.5 Artemia/mL.

Samples of 15 paralarvae from hatchling, control group and experimental group were collected in triplicate from each treatment. Individuals were cold-anaesthetized and homogenized in distilled water. Then the extract was centrifuged and the supernatants used in the different assays. Protein concentration was analyzed according to Bradford (1976) to report the activities per g of protein. The optimal pH for protease activities was determined using Universal Buffer (Stauffer, 1989). Alkaline protease activity was determined according to a modified method described by Sarah et al. (1989) using 1% casein as substrate. Trypsin activity will be determined following the modified method described by Charney and Tomarelli (1947), using BAPNA (Nα-benzoyl-Larginine-4-p-nitroanilide hydrochloride) as substrate. Chymotrypsin activity was assayed according to Delmar et al. (1979), using Succinil-Ala-Ala-Pro-Phe-p-nitroanilide (SAAPNA) as substrate. One unit (U) of activity will be defined as the amount of enzyme liberating 1 µmol of product per min under the conditions described above for each enzymatic assay.

Results and discussion

The optimum pH profiles for protease activities varied markedly and showed a maximum at pH 6.0 for acid proteases and pH 9.0 for alkaline proteases (Figure 1).

Paralarvae from control treatment as well as from LC60 treatment, showed increased total acid and alkaline proteolytic activity levels in comparison with hatchling levels ($P < 0.001$) (Table 1). Chymiotrypsine activity also increased in 12 days old paralarvae ($P <$

0.01) respect to hatchling levels while trypsin activity maintained the hatchling levels (P > 0.5).

On the other hand, acid and alkaline total proteolytic activity of Control and LC60 treatments showed no differences. Trypsine and chymiotrypsine activities were also not different between both experimental groups.

Fig. 1. Effect of pH on **e**nzymatic activity (U mg protein^{-1}) of acid (pH 7-12) and alkaline (pH 3-6) proteases of the *O. vulgaris* paralarvae. Vertical bars show standard deviations.

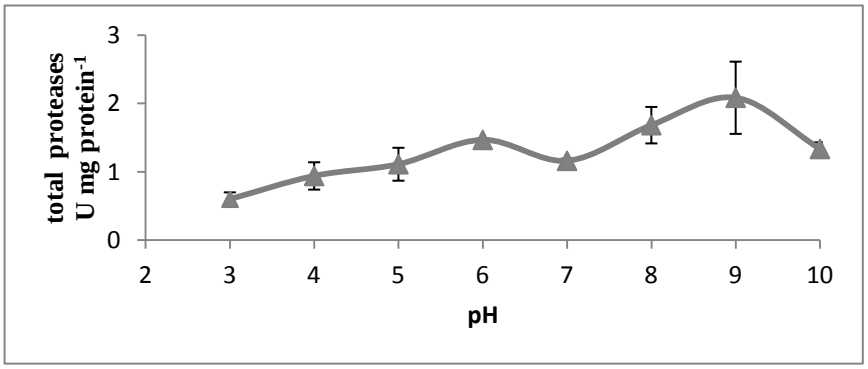

Table 1. Acid and alkaline proteases (U mg protein $^{-1}$), trypsin and chymiotrypsine (mU mg protein $^{-1}$) activities measured at optimum pH in *O. vulgaris* paralarvae.

	Hatchling	Control	LC60
Acid proteases	0.25 ± 0.39 b	1.47 ± 0.02 a	1.62 ± 0.10 a
Alkaline proteases	1.28 ± 0.22 b	1.74 ± 0.13ab	2.08 ± 0.53 a
Chymiotrypsine	14.22 ± 1.14 b	51.01 ± 7.15 a	48.11 ± 16.52 a
Trypsine	8.77 ± 0.70	10.09 ± 0.53	11.06 ± 2.87

Data are presented as means ± S.E.M.

Conclusions

Acid and alkaline proteases and trypsine activities were strongly influenced by paralarvae age. However the presence of marine phospholipids in paralavae diet did not increase the specific enzyme activity of total proteases, trypsin and chymotrypsin.

References

Bradford M.M. 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Annals of Biochemistry 72: 248-254.

Charney J., R. Tomarelli. 1947. A colorimetric method for the determination proteolytic activity of duodenal juice. Biological Chemistry 171: 501-505.

Delmar, E.G., C., Largman, J.W. Brodick and MC. Geokas, 1979. A sensitive new substrate for chymotrysin. Analytical Biochemestry 99: 316-320.

Sarath G., R. S. De La Motte, F.W. Wagner. 1989. Protease assay methods. In: Beynon, R., Bond, J. (Eds.). Proteolytic Enzymes: A Practical Approach. IRL Press, Oxford, pp. 25-56.

Stauffer C.E. 1989. Enzyme Assay for Food Scientists. Van Nostrand Reinhold, New York. USA.

Acknowledgments

This project has received funding from Ministerio de Economia y Competitividad (Spain) through OCTOWELF Project (AGL2013-49101-C2-1-R).